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14. ABSTRACT We are examining the hypothesis that mitochondrial inheritance plays a significant role in aggressiveness of prostate cancer in African Americans. In the first year of the project we have identified 2,000 noncancerous tissues samples from African American men with prostate cancer and we have extracted DNA from ~ 1,500 of them to date. We have validated a robust new DNA sequencing technique developed by our collaborator using single amplicon long-range PCR that permits deep coverage (10,000-20,000X on average) of the mitochondrial genome. We have sequenced 549 samples fully thus far. Mapping of DNA variants in our sequenced genomes to mitochondrial genes has begun and the initial data is presented here. After months of testing, we determined that the ethidium bromide methodology for generating prostate cancer cell line cybrids was not effective and we have instead decided to use the Rhodamine-6-G procedure. Thus far the PC3 and PNT1A cell lines are responding well to this method and we expect to produce cybrids within the next month. We have begun testing additional mitochondrial cell lines in case the LNCaP cell cybrid generation is not successful.

15. SUBJECT TERMS **Mitochondrial DNA sequencing, Prostate cancer, Cybrid cells, African Americans.**

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Introduction:

African American men are disproportionately affected by prostate cancer with increased lifetime risk, earlier onset of disease and more advanced stage at diagnosis than Caucasians. Prostate cancer is the leading cause of cancer death in African American men, with mortality more than double that observed in Caucasians. There are nearly 20 million African American men in the US, many of whom face significant risk of developing and dying from prostate cancer. Risk for and aggressiveness of prostate cancer in African American men is thought to originate in part from genetic susceptibility. Several nuclear genes and chromosomal regions have been linked to prostate cancer; however, many studies have not included African American men, and no study has linked genetic polymorphisms with clinical outcome. One factor which has not been carefully examined is mitochondrial inheritance which varies significantly between ethnic and racial groups and could explain large differences in disease characteristics. In the present study, we are examining the hypothesis that mitochondrial inheritance plays a significant role in aggressiveness of prostate cancer in African Americans. Further, we predict that adverse clinical outcomes will be reflected in dysregulation of cellular biochemical processes and in alterations in signaling pathways (Akt pathway, apoptosis). The ability to identify mitochondrial variants or haplogroups that contribute to aggressive disease will help to separate patients with indolent disease who can be spared unnecessary intervention from those who need more immediate and aggressive therapy. Identification of cellular pathways involved will help to target treatment strategies for those with cancers predicted to be more aggressive. In Aim 1 we will conduct a study of mitochondrial inheritance in 1,000 African American men with prostate cancer. We will sequence the mitochondrial genome of all 1,000 samples and determine whether particular mitochondrial variants, genes or haplogroups are associated with markers of aggressive disease (age at diagnosis, stage at diagnosis (including bone metastasis), Gleason score, PSA at diagnosis, PSA recurrence and death from disease). Findings will be replicated using an independent set of 1,000 patients from our own tissue resource with linked clinical data. Our strategy will be to sequence the mitochondrial DNA of all 1,000 patients in the discovery phase as well as in the replication group. We will control for population admixture using the Illumina African American Admixture Panel. Using cybrid technology, we will introduce our previously identified mt10398A variant and mitochondrial variants associated with highly aggressive and least aggressive disease identified in our genotyping study into prostate cell lines (derived from normal and cancer cells). Cybrids differing only in their mitochondrial composition will be examined for viability under metabolic stress, cell cycle distribution, production of reactive oxygen species, O_2 consumption, ATP synthesis, respiratory chain activity and capacity to grow in an anchorage independent manner. The effect of mitochondrial variants on nuclear gene expression will be studied using Western blotting and microarrays.

Body:

In the text to follow, we provide the Aims and original Statement of Work in italics with progress on the project presented in regular text.

<u>Aim 1:</u> To examine the association between mitochondrial DNA variants and clinical outcome in African American men with *prostate cancer.*

Task 1: <u>Extraction of all DNA for Initial Study and Validation Set</u>

1a. Complete extraction of DNA/ quantitation of DNA for mitochondrial sequencing *Months 1-9*
(Extraction ongoing at present, expect more than 1,000 samples to be prepared before the project is funded. Additional 1,000 to be extracted for validation set IRB protocol already approved)

To date we have extracted DNA on more than 1500 Tissue samples from African American Men with Prostate cancer. Work was initially slower than we had initially planned because of difficulties with extracting adequate DNA from some of the FFPE (paraffin-embedded) samples. We have consulted with colleagues at the University of Arizona for additional insight. Recommendations have been: 1) to extract only 2-3 10 micron sections per tube with 2 tubes per sample to make extraction more effective and increase DNA yield; 2) to allow the ethanol to dry completely off the deparaffin preparation prior to lysis to increase the effectiveness of the lysis; 30 to heat at 90^0C for 2 hours which reducs cross-linking and allows more efficient nucleic acid extraction especially for older specimens. With these modifications we are increasing our yields on smaller volume and older samples.

In the last one year period we have re-extracted some of the FFPE samples from the last period with higher yields and quality. In addition we have obtained additional samples of frozen tissue and of FFPE tissue from prostate cancer patients. We are in the process of amending our IRB submission to allow us to gather more recent tissue samples to increase our sample number to 2,000.

Task 2: Mitochondrial Sequencing

2a.	*Order and test overlapping mitochondrial primers with FFPE DNA to be sure that all primers work with FFPE tissue DNA*	*Ongoing – Month 3*
2b.	*Redesign/test and mitochondrial primers which do not give good PCR results in 2a.*	*Months 4-6*
2c.	*Establish Database for mitochondrial sequences*	*Months 1-5*
2d.	*PCR mitochondrial sequences for primary study and validation set*	*Months 6-20*
2e.	*Sequence mitochondrial PCR products*	*Months 6-20*
	(Illumina Admixture Genotyping to be supported by Helis funds, but performed simultaneously)	*Months 6-20*

As reported in our initial project proposal, based on published sequences for mitochondrial PCR primers[1, 2] we designed 61 pairs of overlapping primers to amplify the entire 16.6-kb mitochondrial genome. In order to test this technology, we used 15 matched DNA samples derived from 5 patients – frozen tissue, FFPE tissue and whole genome amplified DNA samples derived from FFPE DNA for each patient. Overall 2/3 of the amplicons had reads in both directions on all 15 samples (including WGA DNA). Five of 61 amplicons failed completely and will be redesigned. Thus, we covered 93% of the genome by reads in at least one direction in 90% of the samples, and by reads in both directions in 84% of the mt genome, an excellent outcome for a first pass analysis over a target. Using these results, we identified 165 variants as compared to the published mitochondrial sequence. Within these variants there was 96-100% concordance of calls for each patient across the three sample types. We have continued to optimize the primers for the Sanger sequencing protocols, but as we reported in our initial application, we have also continued to test new technologies for mitochondrial sequencing and we are pleased to report here that we have obtained excellent results using an new technique developed by one of the collaborators on the this project (Dr. Lee-Jun Wong). This technique enriches the entire human mitochondrial genome by a single amplicon long-range PCR followed by massively parallel sequencing.[3] This protocol, described briefly below utilizes less than 100 ng of tissue DNA and makes possible a one- step approach to provide quantitative base calls, exact deletion junction sequences and quantification of deletion heteroplasmy. We are not sure that this strategy will be effective in our FFPE DNA samples.

As described in more detail in Zhang et al[3], the forward and reverse primers were: mt16426F (5′-CCGCACAAGAGTGCTACTCTCCTC-3′) and mt16425R (5′-GATATTGATTTCACGGAGGATGGTG-3′). PCR is performed using a TaKaRa LA *Taq* Hot Start polymerase kit (TaKaRa) and 50 ng of total genome DNA isolated from frozen seminal vesicle as template in a 50-μL PCR system. An initial 2-min incubation at 95 °C was followed by 30 cycles of PCR with 20 s of denaturation at 95 °C and 18 min of annealing and extension at 68 °C. The reaction was completed by 1 cycle of final extension at 68 °C for 20 min. Indexed paired-end DNA libraries were prepared according to the manufacturer instructions with minor modifications. Briefly, LR-PCR products were fragmented to 200 bp, which were purified with AMPure XP beads. After end repair, 3′-adenylation, and Illumina InPE adapter ligation, DNA samples were enriched by PCR with Herculase II polymerase (Agilent Technologies). Twelve indexed DNA libraries were pooled together with equal molar ratios. Each pooled library was sequenced in a single lane of 1 flow cell on HiSeq2000 with a 76- or 100-bp paired-end or single-end read chemistry.

Though we are certain that the Sanger sequencing methodology will be effective for our FFPE samples, we are also considering two alternative and potentially more effective strategies for sequencing DNA from these samples. The sequence capture and ION Ampliseq methodologies are described briefly here. Our plan is to compare these alternative methodologies using paired frozen and FFPE tissue DNA derived from the same patient with the long range PCR primer methodology from frozen tissue as our "gold standard".

SEQUENCE CAPTURE: We will use a sequence-specific capture approach followed by Illumina sequencing. For this, we will utilize a custom capture reagent (Roche NimbleGen) targeting the hg19 mitochondrial genome (16.6 Kb). Libraries of ~200 bp insert sizes would be prepared from genomic DNA (500 ng) using HGSC Illumina WES protocol and co-captured at 24-48 plex using full-length blockers to capture reads at higher on-target rates. We have had good success with using this protocol for WES of FFPE samples (4 FFPE samples, co-captured, 9.44 Gb/sample, 20X base coverage of 89. 4%). A total of 96 enriched libraries will be pooled and sequenced on a single Illumina HiSeq 2500 lane (~30 Gb). We estimate that each sample will yield 312 Mb of sequence, which roughly will amount to 8000X depth across the target regions. This is a one third less average coverage to the 12000X coverage data currently generated for frozen tissue DNA samples using the long range PCR protocol, where 80 samples are sequenced per lane on Illumina HiSeq. We expect that duplicate rates, which are usually elevated in small target region captures, will not be an issue in this case, as mitochondrial DNA copy number is 1-2 log orders greater than that of the genomic DNA. Non-specific capture of Pseudogenes in the nuclear genome that share high sequence similarity with functional mtDNA genes is another valid concern when using the above described target enrichment approach. However, recent studies have shown that it is possible to bioinformatically determine the limit on heteroplasmy detection due to such contamination and largely eliminate this concern.[4]

B. ION AMPLISEQ. An alternative approach to be explored is the use of the Ion Ampliseq platform to characterize mitochondrial gene mutations. The Ion AmpliSeq™ technology is an ultra-high multiplex PCR method that requires 10 ng of DNA as input and has been proven to be useful to sequence FFPE samples. A custom amplicon panel will be designed using the Ion AmpliSeq™ Designer software. Care is taken to design amplicons that specifically amplify the hg19 mitochondrial genomic sequences using their 'w*hite glove'* design option that facilitates additional customization to design amplicons for difficult regions. A typical Ampliseq design targeting FFPE samples is expected to be ~ 150 bp, which makes it an ideal size design to sequence on Ion Proton instrument, which currently generates 60-70 Million unidirectional reads. Assuming that there will be 110 amplicons (16.6Kb/150 bp = 110 amplicons) in this mitochondrial gene panel, we can expect an average amplicon coverage of 5600- 6600 X when 96 samples are sequenced in a pool. At BCM-HGSC, we have optimized a high-throughput robotics pipeline for this platform for preparation of libraries quickly and economically and have sequenced over 1200 samples utilizing the Ampliseq Cancer Hotspot Panel v2 and 5 other custom Ampliseq panels.

Figure 1 shows typical results from four samples sequenced by the long range primer technology. We now have sequenced 549 samples using this technology. As seen in figure 1, a distinct advantage provided by amplification of the entire mitochondrial genome by long range PR with a single primer pair is the uniform coverage. This figure demonstrates that we routinely had coverage depth of 10,000-20,000-fold for the mitochondrial genome. This sequencing strategy has multiple advantages including ease of excluding nuclear DNA sequences which are nearly identical to mtDNA and more uniform coverage of the mitochondrial genome because there is much less risk of having a rare or novel variant at the primer binding site when only one set of primers is used. Deep coverage allows much more accurate base calls and improves the limit of detection for heteroplasmy from about 15% with Sanger sequencing to ~ 1-2% with the long range primer technique. As noted above, we expect that this technology will not work as well in the FFPE samples, but we have several viable alternatives. Some question has arisen as to why the depth of coverage is not uniform over the entire mitochondrial genome (sawtooth pattern). We feel this may relate to the GC content of the mitochondrial genome (see figure 1B) which demonstrates a very similar sawtooth pattern, but we do not feel it will affect our overall results.

Figure 1: Mitochondrial DNA sequence data

Figure 1A: Panels A-D show representative coverage for 4 different DNA samples across the entire 16,569 bp mitochondrial genome. The y axis represents fold coverage at each position (x coordinate) along the mitochondrial genome

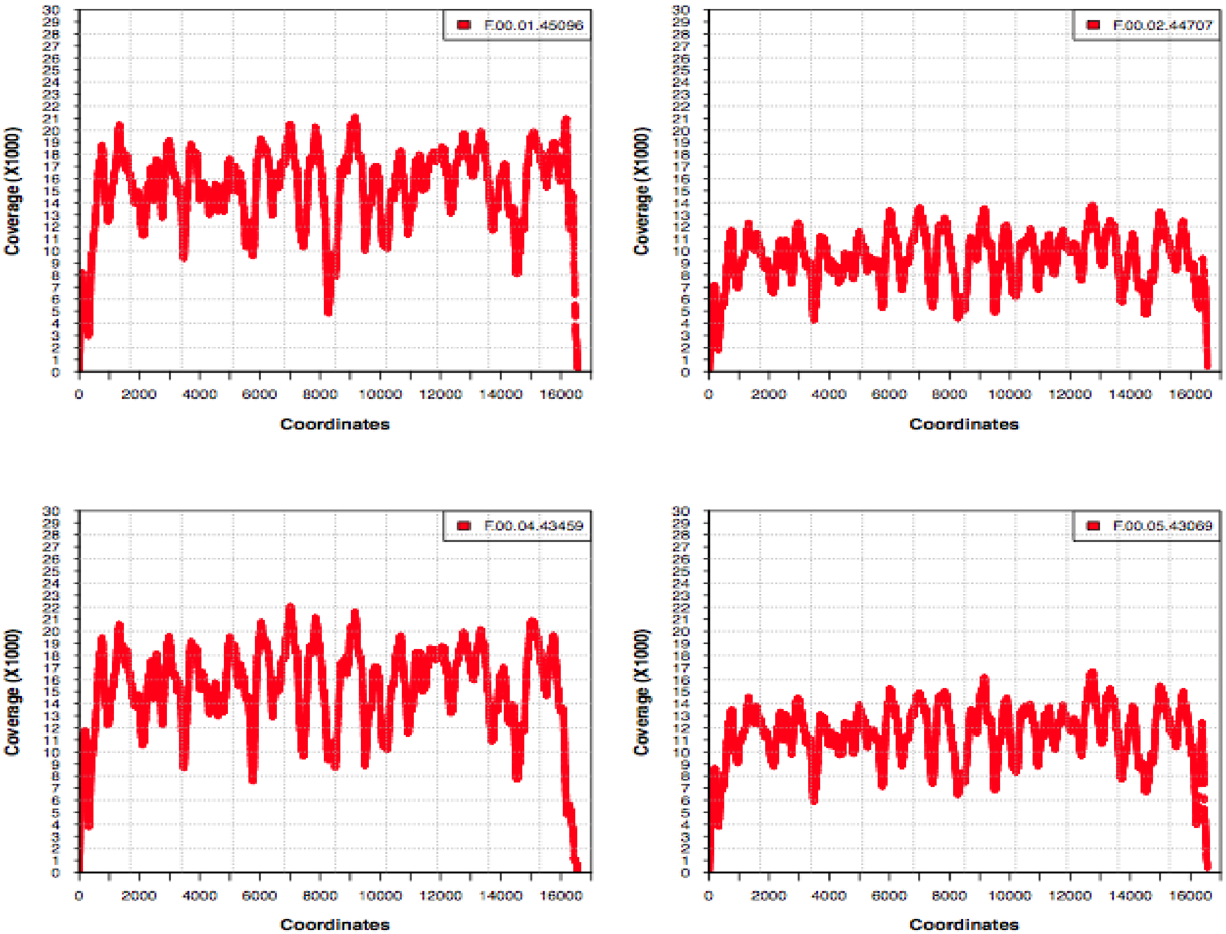
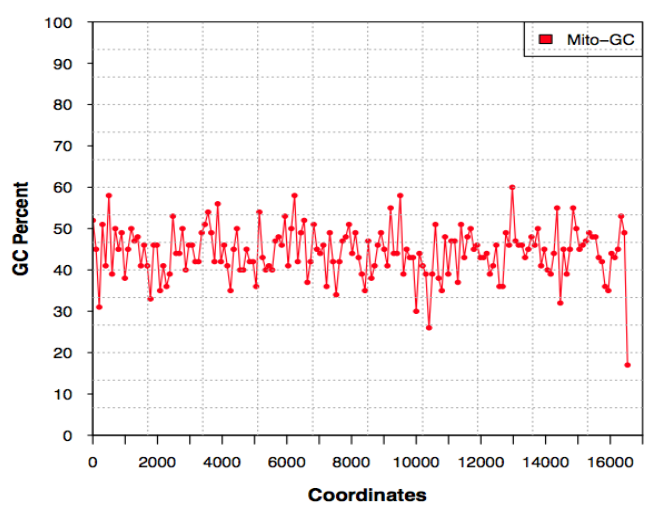


Figure 1B: GC content of the mitochondrial genome



Task 3: <u>Analysis of Mitochondrial Data</u>

3a. Analyze mitochondrial sequences and integrate with clinical data	*Months 20-28*
3b. Admixture analysis based on Illumina data	*Months 20-28*
3c. Identify mitochondrial variants of interest for cybrid study and match with lymphoblast bank	*Months 26-28*

Figure 2A demonstrates the single nucleotide variant (SNV) calls for the first approximately two hundred subjects. On average each subject has about 150 SNVs with the Cambridge mitochondrial sequence used as reference.[5,6] As shown in our previous report, most of the data at each coordinate clustered at 0 or 1. Since the mitochondrial genome is essentially haploid this suggests there there is little contamination with nuclear DNA sequences. Similar data for insertions and deletions is shown in figure 2B.

Figure 2: Single nucleotide variants in the mitochondrial genome for the first sequenced subjects.

Figure 2A: Single nucleotide variants in the mitochondrial genome for the first sequenced subjects.

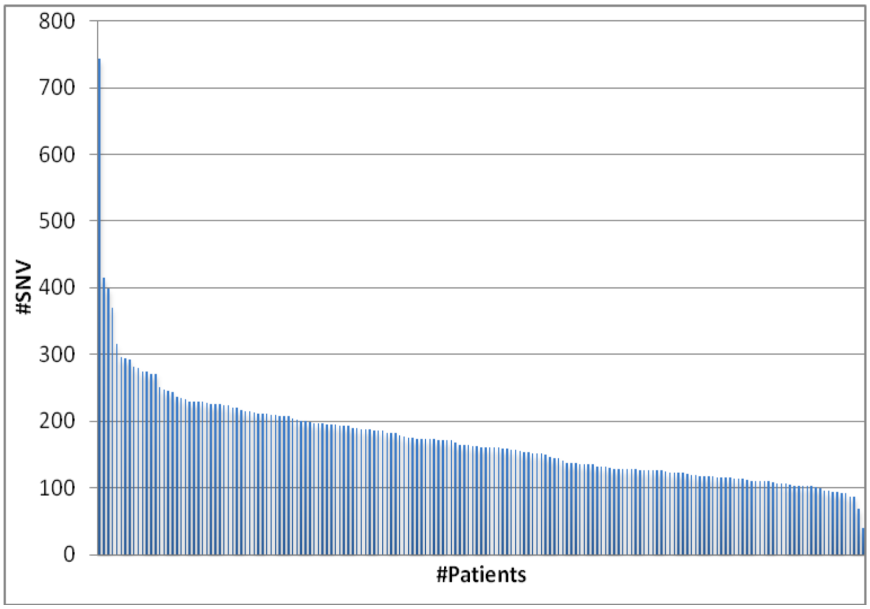
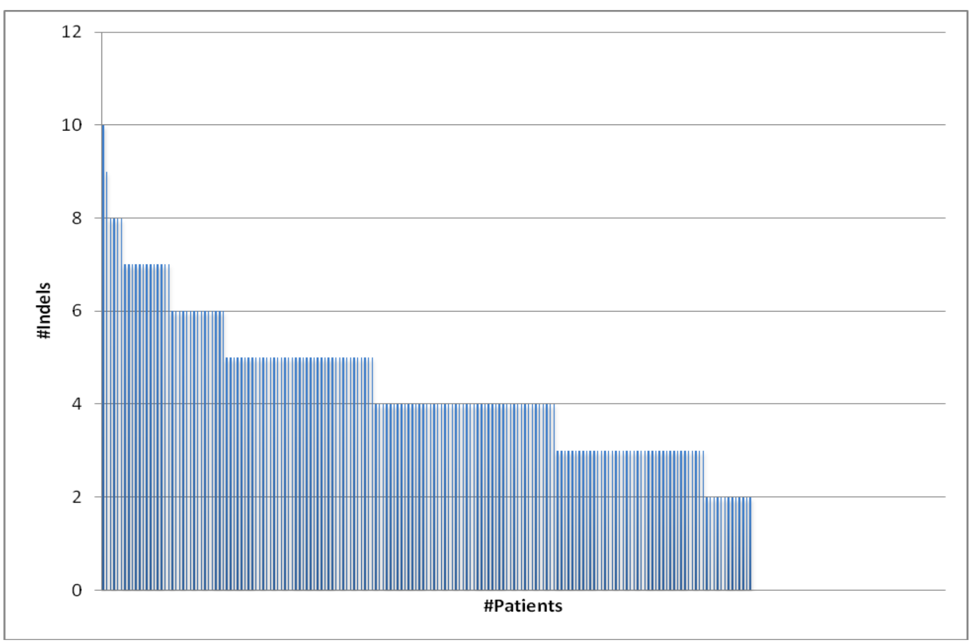


Figure 2B: Insertions and Deletions in the mitochondrial genome for the first sequenced subjects.



We have now created a database of the sequence data which will ultimately include not just mtDNA sequence data, but nuclear SNPs gleaned from the Illumina Race/Ethnicity panel (see below). We have mapped these sequences to the mitochondrial genome (see figure 3 below) to demonstrate the distributions of the mutations found in the various mitochondrial genes. Mutations below the 0.01 variant ratio have been filtered out. Figure 3A shows the SNVs in the mitochondrial genes. As expected the most mutations are seen in the D-loop, but a significant number of additional mutations are found in other regions of the genome as well including the coding regions of for a number of components of the electron transport chain. Similar data in Figure 3B shows the distribution of insertions and deletions across the mitochondrial genes.

Figure 3: Distribution of variants found in mitochondrial genes for the initial subjects.

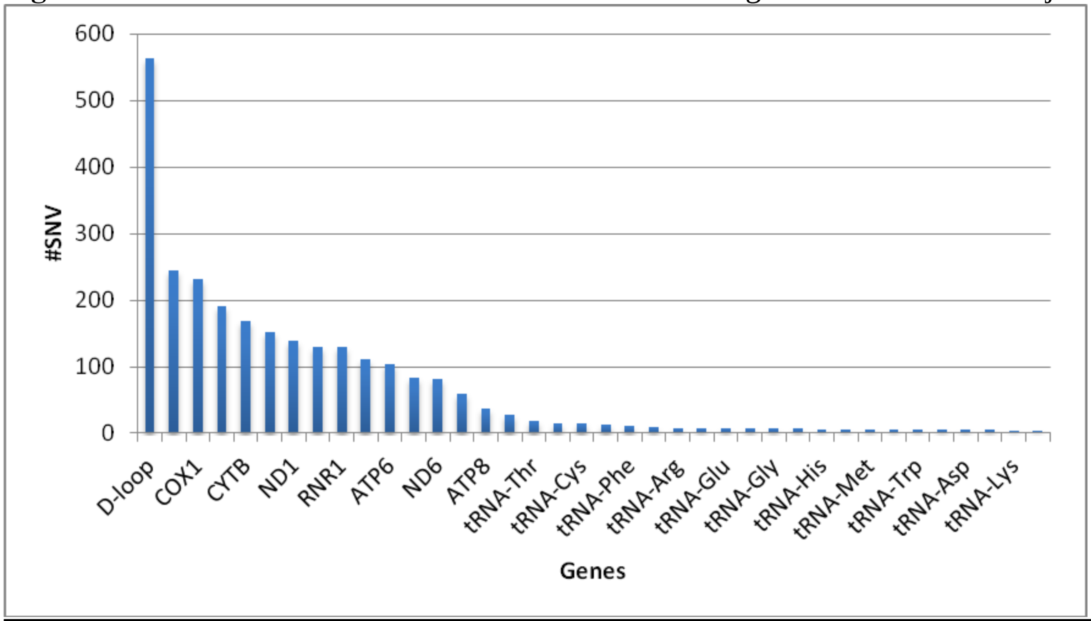


Figure 3A: Distribution of mitochondrial single nucleotide variants found within various mitochondrial genes for the initial subjects as compared to the Cambridge reference sequence. The Y axis represents the absolute number of variants observed within each gene.

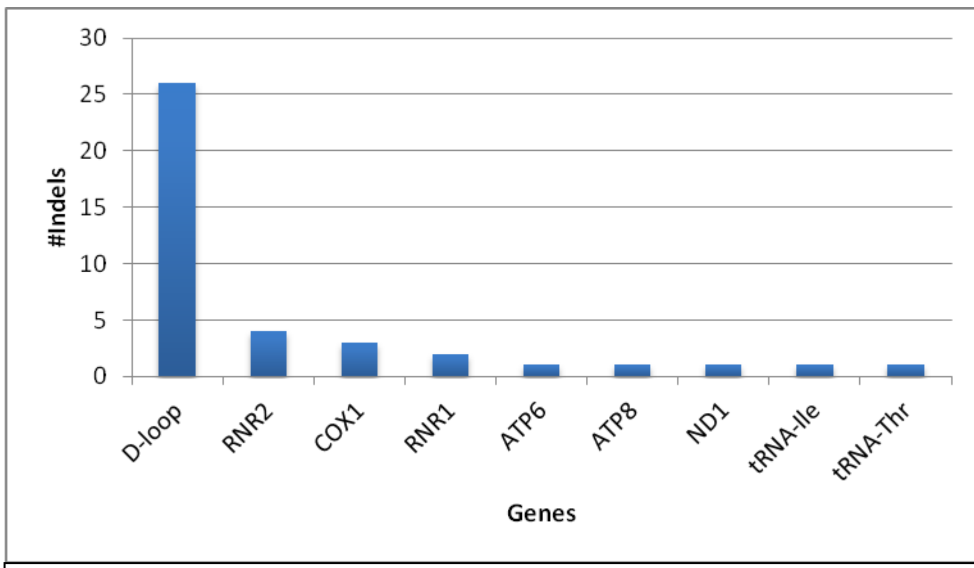


Figure 3B Unique insertions and deletions by gene across the initial patients as compared to the Cambridge reference sequence. The Y axis represents the absolute number of insertions/deletions observed within each gene.

Although not covered by this project, we have also sent the first 72 samples for genotyping on the Illumina African American Admixture panel. All 72 samples were successfully genotyped.

<u>Aim 2:</u> To understand the role of mitochondrial DNA variants in regulation of cellular processes important in the cancer cell including those involved with generation of reactive oxygen species and energy metabolism.

Task 1: <u>Creation of Cybrid cell lines</u>

1a.	*IRB submission for creation of lymphoblastoid lines from patients*	*Months 1-4*
1b.	*Creation of immortalized lymphocyte bank for use in study*	*Months 5-24*
1c.	*Order/test primers for variable number tandem repeat studies of nuclear origin*	*Months 6-12*
1d.	*Deplete prostate cancer cells of mitochondria 3 potential cell lines to be used: PNT1A, LNCaP or PC3*	*Months 9-24*
1e.	*Create cytoplasts and fuse with prostate cancer cells devoid of mitochondria to create cybrid cell lines*	*Months 20-36*
1f.	*Confirm nuclear origin of cybrid cell lines using variable length tandem repeat in insulin receptor and other genes*	*Months 20-36*

In collaboration with Dr. Michael Ittmann, the IRB submission for creation of lymphoblastoid cell lines was obtained before the project was actually funded. We have been in discussion with the HRPO regarding approval of the IRB protocols and have obtained full approval (see appendix) of both protocols required for our studies. With assistance from Dr. Ittmann, we are obtaining blood for creation of the lymphoblastoid cell lines and we have begun the initial steps in creating lymphoblastoid cell lines.

We have begun to make mitochondria-depleted Rho^0 cells from prostate cancer cell lines PC3, LNCaP and PNT1A. These cells were treated with Ethidium Bromide (EB) at varying concentrations. To confirm mitochondrial DNA (mtDNA) depletion, the mtDNA content was intermittently checked by qPCR analysis. Initially, among the three cell lines, the mtDNA copy number decreased significantly in benign PNT1A cells as compared to the PC3 and LNCaP. The dosage of EB for the cancer cells PC3 and LNCaP was increased to varying concentrations. Though the mtDNA content in PNT1A cell lines decreased considerably, mtDNA persisted without the support of external uridine. Since this condition is not enough for the cybrid generation, the EB treatment was continued for all the cells at varying concentrations. Though we observed reduction on mtDNA copy number, over time the cells gained resistance to EB. Despite multiple attempts, this problem persisted and has been reported previously in several cell lines. Even though the benign cell line PNT1A showed a significant and consistent decrease in mtDNA copy number, it did not reach complete depletion of mtDNA and the cells were able to grow in the absence of exogenous uridine. Since dependency on exogenous uridine is a prerequisite for selection of transmitochondrial cybrids clones, none of the cell lines achieved the required criteria for nuclear donors in cybrid generation.

Since mitochondrial DNA depletion by the EB method failed to give Rho^0 cells with required depletion of mtDNA, we opted for an alternative method utilizing Rhodamine6G (R6G) treatment for cybrid generation. The prostate cell lines were treated with different concentrations of R6G ranging from 3 μg/ml to 1μg/ml for a short period of time. We expect gradual death of cells after R6G treatment if the cells are not fused with mitochondria from donor cells. Using this method, we fused mitochondria from different cells including PC3, LNCaP and PNT1A. Though we generated several single cell clones, detailed sequencing of mtDNA revealed that these clones contained a significant amount of mtDNA from the nuclear donor. We have titrated the dose of Rhodamine and duration of treatment and repeated the experiments. We have recently demonstrated a positive response on colonies with PNT1A nuclear background. These single colonies require further expansion as well as confirmation by sequencing of mtDNA and analysis of cellular properties. Based on these positive responses, we are performing cybrid fusion with varying doses and duration of Rhodamine treatment. Thus far PC3 and PNT1A are responding well to this method and we expect to produce cybrid cells using these lines in the next few months. LNCaP cells easily undergo apoptosis when exposed to Rhodamine under these conditions and we are uncertain that we can achieve cybrid cells using LNCaP cells, thus we have begun testing a series of less aggressive prostate cancer cell lines to replacae LNCaP cells if we are unsuccessful. These additional cell lines are currently undergoing testing and include: VCaP, 22RV1 and PC346c, DU145 and LAPC4. Considering the results over the past several months, we expect a positive outcome for PC3 and PNT1A cells and we feel confident that either LNCaP cells or one of the other cell lines will also provide positive results.

Since a drug resistant gene is necessary in the nuclear donor cells for the selection of cybrids after fusion, we have generated puromycin resistant strains of all the cells via transfection. The stable resistant clones with puromycin resistant genes are being used for the dose optimization experiments. Also as a pre-requisite for cybrid clone confirmation, we are also sequencing the DNA from mitochondrial and nuclear donor cells for mitochondrial and nuclear DNA mutations by Next Generation Sequencing (NGS) so that we can confirm the origin of the cybrids after cellular fusion. We feel that sequencing techniques are rapidly the need to use variable number of tandem repeat studies to test nuclear origin.

Key Accomplishments:

1. Isolation of more than 90% of the DNA samples required for the entire project.
2. Validation of a more robust sequencing technique using single amplicon long-range PCR that permits deep coverage (10,000-20,000X on average) of the mitochondrial genome, to date the first 549 samples have been sequenced.
3. Mapping of the DNA variants to mitochondrial genes (data shown for ~first 200 samples).
4. Illumina African Admixture mapping of the initial 75 subjects demonstrating that the use of FFPE DNA for these studies is feasible:
5. Strategy for creating cybrids using prostate cancer cell lines has been confirmed using Rhodamine 6G. necessary for cybrid generation –
    a. puromycin resistant cell lines have been created for all cell lines
    b. puromcyin resistant cells treated with Rhodamine6G to deplete the cells of mitochondria has resulted in creation of first few colonies of PC3 and PNT1A cells cured of mitochondria. Additional cell lines being tested in case Rho^0 cells cannot be created from the LNCaP cell line. Conditions for each of these strategies are being optimized.

Reportable Outcomes:

No completed reportable outcomes have been accomplished as yet, but cybrid cell lines may soon be available. We are in the process of creating the Mitochondrial database with mtDNA sequence data, African American Admixture Data and Clinical Data.

Conclusions: Thus far we have made significant progress toward mitochondrial sequencing of the 2000 subject samples required for this project. In concert with Dr. David Wheeler in the Human Genome Sequencing Center and Dr. Lee-Jun Wong, we have implemented a new more rapid and much more powerful technique for sequencing mitochondrial DNA using single amplicon long-range PCR. This technology has permitted us to rapidly sequence a large number of samples using NGS technology at a depth of coverage of 10,000-20,000X. This technology allows much more accurate base calls and improves the limit of detection for heteroplasmy from about 15% with Sanger sequencing to ~ 1-2% with the long range primer technique. Nearly six hundred DNA samples are now in the sequencing pipeline. This technology has permitted much more rapid generation of mtDNA sequence with much less DNA and less "contamination" by nuclear DNA. We are hopeful that this technology will be applicable to the FFPE DNA once optimized, but we are continuing to work on additional strategies for the FFPE samples using accurate high throughput technology. We have initiated the genotyping for Admixture mapping which required only 25 ng of DNA – much less DNA than we had initially thought would be required. Finally though we have had to alter our strategy, we have made significant progress in creating the cybridcells which will be required for our experiments. To date we have created the puromycin resistant cell lines and we have optimized the Rhodamine strategy for generating Rho^0 cells from PNT1A and

PC3 cells and initial colonies have been generated. LNCaP Rho^0 cells have proven more difficult to generate, but we have alternative strategies using different cell lines underway which should shortly yield results.

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APPENDIX

Mims, Martha Pritchett

From:	Odam, Kimberly L CIV USARMY MEDCOM USAMRMC (US) [kimberly.l.odam.civ@mail.mil]
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Cc:	Bennett, Jodi H CIV USARMY MEDCOM USAMRMC (US); Drake, Carrie E CTR USARMY MEDCOM (US); Katopol, Kristen R CTR USARMY MEDCOM (US); Mims, Martha Pritchett; Ellison, Mirlene D CIV USARMY MEDCOM USAMRAA (US); Miller, Theresa J CTR USARMY MEDCOM CDMRP (US); Brosch, Laura R CIV USARMY MEDCOM USAMRMC (US); Shank, Patricia A CTR USARMY MEDCOM USAMRMC (US); King, Darlene; Odam, Kimberly L CIV USARMY MEDCOM USAMRMC (US)
Subject:	A-17746.2 HRPO Approval Memorandum (IRB Study Number H-17002, Proposal Log Number PC101454, Award Number W81XWH-11-1-0737) (UNCLASSIFIED)

Classification: UNCLASSIFIED
Caveats: NONE

SUBJECT: Initial Approval for the Protocol, "Molecular Correlates of Prostate Cancer Risk and Outcome," Submitted by Michael M. Ittmann, MD, PhD, Baylor College of Medicine, Houston, Texas, in Support of the Proposal, "Role of Mitochondrial Inheritance on Prostate Cancer Outcome in African American Men," Submitted by Martha P. Mims, MD, Baylor College of Medicine, Houston, Texas, IRB Study Number H-17002, Proposal Log Number PC101454, Award Number W81XWH-11-1-0737, HRPO Log Number A-17746.2

1. The subject protocol, amended to include the DOD-funded research, was approved by the Baylor College of Medicine Institutional Review Board (IRB) on 8 May 2013 for the engagement of the Baylor College of Medicine and the Michael E. Debakey Veterans Affairs Medical Center (MEDVAMC). This protocol was reviewed by the US Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) and found to comply with applicable DOD, US Army, and USAMRMC human subjects protection requirements.

2. This no greater than minimal risk study is approved to identify tissues and data with appropriate clinical and pathological characteristics from up to 2000 specimens from subjects treated at the MEDVAMC. DNA extraction and analysis will be performed at the Baylor College of Medicine.

3. The Principal Investigator has a duty and responsibility to foster open and honest communication with research subjects. The USAMRMC strongly encourages the Principal Investigator to provide subjects with a copy of the research protocol, if requested, with proprietary and personal information redacted as needed.

4. The following are reporting requirements and responsibilities of the Principal Investigator to the HRPO. **Failure to comply could result in suspension of funding.**

a. Substantive modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the HRPO for approval prior to implementation. The USAMRMC ORP HRPO defines a substantive modification as a change in Principal Investigator, change or addition of an institution, elimination or alteration of the consent process, change to the study population that has regulatory implications (e.g. adding children, adding active duty population, etc.), significant change in study design (i.e. would prompt additional scientific review), or a change that could potentially increase risks to subjects.

b. All unanticipated problems involving risk to subjects or others must be promptly reported by telephone (301-619-2165), by email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), or by facsimile (301-619-7803) to the HRPO. A complete written report will follow the initial notification. In addition to the methods above, the complete report can be sent to the US Army Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000.

c. Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the sponsor, or regulatory agencies will be promptly reported to the USAMRMC ORP HRPO.

d. Events or protocol reports received by the HRPO that do not meet reporting requirements identified within this memorandum will be included in the HRPO study file but will not be acknowledged.

e. A copy of the continuing review approval notification by the Baylor College of Medicine IRB must be submitted to the HRPO as soon as possible after receipt of approval. According to our records, it appears the next continuing review by the Baylor College of Medicine IRB is due no later than 8 October 2013. Please note that the HRPO conducts random audits at the time of continuing review and additional information and documentation may be requested at that time.

f. The final study report submitted to the Baylor College of Medicine IRB, including a copy of any acknowledgement documentation and any supporting documents, must be submitted to the HRPO as soon as all documents become available.

g. The knowledge of any pending compliance inspection/visit by the Food and Drug Administration (FDA), Office for Human Research Protections, or other government agency concerning this clinical investigation or research; the issuance of inspection reports, FDA Form 483, warning letters, or actions taken by any regulatory agencies including legal or medical actions; and any instances of serious or continuing noncompliance with the regulations or requirements must be reported immediately to the HRPO.

5. **Please note**: The USAMRMC ORP HRPO conducts site visits as part of its responsibility for compliance oversight. Accurate and complete study records must be maintained and made available to representatives of the USAMRMC as a part of their responsibility to protect human subjects in research. Research records must be stored in a confidential manner so as to protect the confidentiality of subject information.

6. Do not construe this correspondence as approval for any contract funding. Only the Contracting Officer/Grants Officer can authorize expenditure of funds. It is recommended that you contact the appropriate contract specialist or contracting officer regarding the expenditure of funds for your project.

7. The HRPO point of contact for this study is Patricia A. Shank, BSN, RN, CCRP, PMP, Human Subjects Protection Scientist, at 301-619-2282/email: patricia.a.shank7.ctr@mail.mil.

KIMBERLY L. ODAM, MS, CIP
Human Subjects Protection Scientist
Human Research Protection Office

Office of Research Protections
US Army Medical Research and Materiel Command

Note: The official copy of this memo is housed with the protocol file at the Office of Research Protections, Human Research Protection Office, 810 Schreider Street, Fort Detrick, MD 21702-5000. Signed copies will be provided upon request.

Classification: UNCLASSIFIED
Caveats: NONE

Mims, Martha Pritchett

From: Brosch, Laura R CIV USARMY MEDCOM USAMRMC (US) [laura.r.brosch.civ@mail.mil]
Sent: Monday, July 29, 2013 9:52 AM
To: Mims, Martha Pritchett
Cc: Bennett, Jodi H CIV USARMY MEDCOM USAMRMC (US); Drake, Carrie E CTR USARMY MEDCOM (US); Ittmann, Michael M; Katopol, Kristen R CTR USARMY MEDCOM (US); Ellison, Mirlene D CIV USARMY MEDCOM USAMRAA (US); Miller, Theresa J CTR USARMY MEDCOM CDMRP (US); Brosch, Laura R CIV USARMY MEDCOM USAMRMC (US); King, Darlene; Shank, Patricia A CTR USARMY MEDCOM USAMRMC (US)
Subject: A-17746.1 HRPO Approval Memorandum (IRB Study Number H-30862, Proposal Log Number PC101454, Award Number W81XWH-11-1-0737) (UNCLASSIFIED)

Classification: UNCLASSIFIED
Caveats: NONE

SUBJECT: Initial Approval for the Protocol, "Molecular and Genetic Studies in Patients With Solid Tumors," Submitted by Martha P. Mims, MD, Baylor College of Medicine, Houston, Texas, in Support of the Proposal, "Role of Mitochondrial Inheritance on Prostate Cancer Outcome in African American Men," IRB Study Number H-30862, Proposal Log Number PC101454, Award Number W81XWH-11-1-0737, HRPO Log Number A-17746.1

1. The subject protocol, inclusive of DOD requested revisions, was approved by the Baylor College of Medicine Institutional Review Board (IRB) on 12 July 2013 on behalf of the Baylor College of Medicine and the Harris County Hospital District Ben Taub General Hospital. This protocol was reviewed by the US Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) and found to comply with applicable DOD, US Army, and USAMRMC human subjects protection requirements.

2. This no greater than minimal risk study is approved for the enrollment of 500 subjects across all study sites.

3. The Principal Investigator has a duty and responsibility to foster open and honest communication with research subjects. The USAMRMC strongly encourages the Principal Investigator to provide subjects with a copy of the research protocol, if requested, with proprietary and personal information redacted as needed.

4. The following are reporting requirements and responsibilities of the Principal Investigator to the HRPO. **Failure to comply could result in suspension of funding.**

a. Substantive modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the HRPO for approval prior to implementation. The USAMRMC ORP HRPO defines a substantive modification as a change in Principal Investigator, change or addition of an institution, elimination or alteration of the consent process, change to the study population that has regulatory implications (e.g. adding children, adding active duty population, etc.), significant change in study design (i.e. would prompt additional scientific review), or a change that could potentially increase risks to subjects.

b. All unanticipated problems involving risk to subjects or others must be promptly reported by telephone (301-619-2165), by email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), or by facsimile (301-619-7803) to the HRPO. A complete written report will follow the initial notification. In addition to the methods above, the complete report can be sent to the US Army Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000.

c. Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the sponsor, or regulatory agencies will be promptly reported to the USAMRMC ORP HRPO.

d. Events or protocol reports received by the HRPO that do not meet reporting requirements identified within this memorandum will be included in the HRPO study file but will not be acknowledged.

e. A copy of the continuing review approval notification by the Baylor College of Medicine IRB must be submitted to the HRPO as soon as possible after receipt of approval. According to our records, it appears the next continuing review by the Baylor College of Medicine IRB is due no later than 28 March 2014. Please note that the HRPO conducts random audits at the time of continuing review and additional information and documentation may be requested at that time.

f. The final study report submitted to the Baylor College of Medicine IRB including a copy of any acknowledgement documentation and any supporting documents, must be submitted to the HRPO as soon as all documents become available.

g. The knowledge of any pending compliance inspection/visit by the Food and Drug Administration (FDA), Office for Human Research Protections, or other government agency concerning this clinical investigation or research; the issuance of inspection reports, FDA Form 483, warning letters, or actions taken by any regulatory agencies including legal or medical actions; and any instances of serious or continuing noncompliance with the regulations or requirements must be reported immediately to the HRPO.

5. **Please note**: The USAMRMC ORP HRPO conducts site visits as part of its responsibility for compliance oversight. Accurate and complete study records must be maintained and made available to representatives of the USAMRMC as a part of their responsibility to protect human subjects in research. Research records must be stored in a confidential manner so as to protect the confidentiality of subject information.

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7. The HRPO point of contact for this study is Patricia A. Shank, BSN, RN, CCRP, PMP, Human Subjects Protection Scientist, at 301-619-2282/email: patricia.a.shank7.ctr@mail.mil.

LAURA R. BROSCH, RN, PhD
Director, Office of Research Protections
Director, Human Research Protection Office
US Army Medical Research and Materiel Command

Note: The official copy of this memo is housed with the protocol file at the Office of Research Protections, Human Research Protection Office, 810 Schreider Street, Fort Detrick, MD 21702-5000. Signed copies will be provided upon request.

Classification: UNCLASSIFIED
Caveats: NONE